

**Life's matrix:
Water in the cell**

Philip Ball

Nature

4 Crinan St

London N1 9XW

UK

p.ball@nature.com

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Water is a profoundly unusual liquid, and its peculiarities may make it uniquely suited to act as life's matrix. Even if this were not so, however, we should expect the effects of nanometre-scale confinement and inhomogeneities owing to surface effects to alter the liquid's properties in the cell relative to those in the bulk. Whether water's unusually high degree of local structure makes such influences even more marked than for 'normal' liquids remains an open question, with potentially important consequences for biomolecular interactions.

Eccentric behaviour seems normal unless one has a basis for comparison. Thus cell biologists, who generally know of no other medium but the aqueous cytoplasm, can hardly be expected to appreciate what a profoundly strange and perplexing liquid water is.¹ Only from the perspective of the liquid-state theory developed within condensed-matter physics does it become apparent how water breaks so many rules that it needs to be considered in a class of its own.

This in itself need not trouble those who study water's role as transport medium, lubricant and active participant in the biochemistry of the cell. Why should it matter that water is unique, if water is the only liquid on the menu? But the reasons for water's eccentricity are also the reasons why its behaviour should not blithely be extrapolated from the bulk to the microscopic scale of the cell. Within water's unusual physical properties there may be profound implications for the factors that influence biomolecular interactions.

It is for this reason that we would be wise to consider water as a biomolecule in its own right, and worthy of study as such. Albert Szent-Gyorgyi's description of it as the 'matrix of life' should not tempt us to regard it as a mere backdrop on which life's tapestry is embroidered. Instead, the words of Gerstein and Levitt in a recent article give a better pointer:

When scientists publish models of biological molecules in journals, they usually draw their models in bright colors and place them against a plain, black background. We now know that the background in which these molecules exist - water - is just as important as they are.²

Water's anomalies

The one thing all biochemists learn about water is that it forms hydrogen bonds. To the physicists, this immediately makes it an unrepresentative liquid. Conventional liquid-state theory can be regarded as a kind of perturbative approximation to the kinetic theory of gases. Simply by taking account of the mutual molecular attractions (the 'internal pressure') in a dense fluid, and the 'excluded volume' owing to finite molecular size, J. D. van der Waals in the nineteenth century developed a theory of the liquid and gaseous states and the transition between them.³ In effect this amounted to the inclusion of the attractive and repulsive intermolecular interactions that the kinetic theory, with its point-like particles, neglected. Liquid-state theory now commonly invokes subtler manifestations of these forces: a short-ranged repulsion that increases steeply with diminishing separation, and a longer-ranged attraction that falls off monotonically with distance.⁴

The critical question pertaining to solvent effects on intermolecular interactions of solutes is then how the solvent is structured. For a 'normal' liquid, there is negligible ordering beyond a few molecular diameters of any particular solvent or solute molecule: the liquid is homogenous and uniform at such distances. Short-ranged ordering, meanwhile, amounts to a series of a few fuzzy solvation shells whose disposition is dominated by the repulsive interactions - in other words, by considerations of how hard-sphere-like solvent molecules can pack together.

For water, it is a different story. The strong and directional nature of the hydrogen bond makes the attractive forces the dominant factor in determining short-ranged order. These bonds tend to localize the hydrogen atom of one H₂O molecule at a distance from the oxygen atom of another that is about twice the intramolecular O-H separation, and to disport around each H₂O molecule a quartet of others in a tetrahedral arrangement (Figure 1). This tetrahedral motif recurs repeatedly throughout an extended, three-dimensional hydrogen-bonded network.⁵ But this framework is constantly rearranging dynamically on a picosecond timescale in the liquid, and is consequently very far from the perfectly regular structure found in ice.

The short-ranged ordering of the hydrogen-bonded tetrahedra prevents the molecules from moving as close to one another as they would in a liquid that did not exhibit these interactions - the network is a very open structure. This is the key to most of water's anomalies, such as its expansion on freezing and its density maximum at 4 °C. For example, the dynamism that permits some degree of closer intermolecular approach in the liquid is prohibited in ice, so that the crystalline solid contains more void space. For many purposes, the structure of water can be regarded as a compromise between this low-density ice-like form and a higher-density form that shares more in common with 'normal' liquids. Under very high pressures and low temperatures, this conflict has been proposed as a cause of a possible phase separation into two distinct (deeply metastable) liquid states.⁶

Hydrophobic effects

The infinite hydrogen-bonded network has given rise to the idea of water as a highly structured liquid. This clearly has some validity, but has also created misconceptions. The influence of the 'iceberg' model of hydrophobic hydration, due to Henry Frank and Marjorie Evans in 1945⁷, is still surprisingly pervasive, though undoubtedly over-simplistic. In their own words,

When a rare gas atom or nonpolar molecule dissolves in water at room temperature it modifies the water structure in the direction of greater crystallinity - the water, so to speak, builds a microscopic cage around it.⁷

The idea is that this increase in structure prevents hydrogen-bond breaking and thus loss of enthalpic stabilization. But there is an entropic cost to the rearrangement, which (the story goes) is the origin of hydrophobic attraction. When two hydrophobic particles aggregate, their shared interface decreases the amount of 'structured' water that surrounds them, and so there is an increase in entropy.

This entropic argument, due to Walter Kauzmann in 1959,⁸ is still routinely peddled; but there is no strong evidence for enhanced structuring of water around hydrophobic solutes. Most of the studies have employed either neutron scattering or probes of local structure such as EXAFS,⁹ but they are hampered by the fact that the low solubility of hydrophobes such as krypton or methane make it hard to sequester a clear signal. The indications are that there may be orientational but not positional order in the solvation shell: the O-H bonds of the water molecules become preferentially aligned tangential to the solute particle. The free-energy change that creates the hydrophobic interaction thus may have other, more subtle, origins.¹⁰

Given the indisputable importance of hydrophobic interactions in cell biology - the driving force for lipid self-assembly into membranes, for instance, and a major determinant of protein structure - these issues are of more than incidental interest. So too is the question (to some extent related) of how water is structured close to both hydrophilic and hydrophobic surfaces.

One thing is clear: the water that intervenes between two proteins, or two lipid bilayers, separated by just a few nanometres cannot be expected to behave as it does in the bulk. There is scant acknowledgement of this fact in the biological literature, but it is uncontroversial in liquid-state physics.

Water is not unusual in this respect. Any liquid confined between surfaces on a scale comparable to a few molecular diameters has a structure which deviates from that of the bulk.¹¹ At these scales, the same packing effects that create solvation shells come into play to promote a layer-like ordering parallel to the surfaces. This ordering is evident in the sawtooth oscillations of the force-distance relationship between two surfaces, as measured in the surface force apparatus for water and other liquids,¹² as well as being found in simulations¹³ and theory.¹⁴

But for water there is the possibility that the unusual degree of short-ranged structure in the bulk liquid will modify this ordering due to confinement. It has even been proposed that this is the cause of the remarkable and still mysterious long-ranged hydrophobic attraction between hydrophobic surfaces in water, which can extend over distances of about 300 nm, or a thousand or so molecular diameters.¹⁵ Yet it is hard to see how even water could maintain any appreciable structuring over such scales, and the most likely explanation at present seems to be that sub-microscopic bubbles of dissolved gas are nucleated between the surfaces,¹⁶ in much the same way as 'capillary drying' can take place in a confined liquid above the saturated vapour pressure.¹⁷ Needless to say, there is no lack of dissolved gases in cells.

Such long-ranged effects aside, it seems most unlikely that the water around two approaching protein molecules will continue to behave like the bulk liquid up to the point where one binds the other. The traditional biochemical concept of 'bound' and 'free' water serves as a convenient shorthand, but surely cannot do justice to the full complexity of behaviour in the two solvation shells. The question of whether such effects have a significant influence on either the kinetics or the thermodynamics of such macromolecular interactions is one that no impartial observer can yet answer. The advent of single-molecule probe techniques such as the atomic force microscope promises, however, to provide some exciting insights.¹⁸

Thus one can find the relevance of water's unusual liquid-state structure both advocated in and dismissed from biomolecular interactions. Israelachvili and Wennerstrom, for example, have argued that at least as far as hydrophilic surfaces are concerned, "as a solvent and suspending medium, water should be seen as an ordinary liquid".¹⁹ At the other extreme, Wiggins suggests that changes in water's local density owing to its proximity to surfaces in the cell might alter profoundly its solvation properties: as the water becomes more structured and ice-like, it is a poorer solvent for ions.²⁰ This could lead to steep concentration gradients, with strong associated osmotic pressures.

Water as a biomolecule

Protein crystal-structure determinations have made it plain that some water is essentially impounded from the solvent for incorporation as a kind of modular extension of the macromolecules. For example, water trapped as hydrogen-bonded chains in protein channels seems able to act as a kind of proton wire,²¹ transferring protons rapidly via the Grothuss process of hydrogen-bond flipping.²² This can facilitate the delivery of protons to a catalytically active site, for example in cytochromes.²³ Transient proton wires have been implicated in the leakage of protons across cell membranes.²⁴ Royer et al. have reported evidence that water molecules bound at the interface of the dimeric haemoglobin of *Scapharca* act as transmission units for allosteric effects that promote the cooperativity of oxygen binding.²⁵ And Quioco et al. have shown how water in the binding pocket of bacterial arabinose-binding protein provides a part of the jigsaw that enables the protein to bind L-arabinose selectively.²⁶ Remarkably, such delicate shape selectivity towards sugars seems even to operate in the free liquid: Galema et al. suggest that the

commensurability of the hydrogen-bonding groups of D-galactose with water's hydrogen-bonded network is responsible for its greater hydrophilicity than D-talose.²⁷

These are not isolated examples. As our understanding of the nature of cell water unfolds, there will surely be an increasing case to argue for a genuine 'biology of water'. The first step, however, is to broadcast the message that there are important questions here that still beg answers. My own belief is that, as we probe deeper into the details of biomolecular structure, dynamics and interactions, this message will become impossible to ignore.

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Figure caption

Fig. 1 The tetrahedral hydrogen-bonding arrangement in liquid water. On average, each H_2O molecule adopts this configuration; but the network is highly fluxional and defective.